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Ambient $PM_{2.5}$ and specific sources increase inflammatory cytokine responses to stimulators and reduce sensitivity to inhibitors

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ABSTRACT

Ambient exposure to fine particulate matter ($PM_{2.5}$) is associated with increased morbidity and mortality from multiple diseases. Recent observations suggest the hypothesis that trained immunity contributes to these risks, by demonstrating that ambient $PM_{2.5}$ sensitizes innate immune cells to mount larger inflammatory response to subsequent bacterial stimuli. However, little is known about how general and durable this sensitization phenomenon is, and whether specific sources of $PM_{2.5}$ are responsible. Here we consider these issues in a longitudinal study of children. The sample consisted of 277 children (mean age 13.92 years; 63.8% female; 38.4% Black; 32.2% Latinx) who completed baseline visits and were re-assessed two years later. Fasting whole blood was *ex vivo* incubated with 4 stimulating agents reflecting microbial and sterile triggers of inflammation, and with 2 inhibitory agents, followed by assays for IL-1β, IL-6, IL-8, and TNF-α. Blood also was assayed for 6 circulating biomarkers of low-grade inflammation: C-reactive protein, interleukin-6, -8, and -10, tumor necrosis factor-α, and soluble urokinase-type plasminogen activator receptor. Using machine learning, levels of 15 p.m.$_{2.5}$ constituents were estimated for a 50 m grid around children's homes. Models were adjusted for age, sex, race, pubertal status, and household income. In cross-sectional analyses, higher neighborhood $PM_{2.5}$ was associated with larger cytokine responses to the four stimulating agents. These associations were strongest for constituents released by motor vehicles and soil/crustal dust. In longitudinal analyses, residential $PM_{2.5}$ was associated with declining sensitivity to inhibitory agents; this pattern was strongest for constituents from fuel/biomass combustion and motor vehicles. By contrast, $PM_{2.5}$ constituents were not associated with the circulating biomarkers of low-grade inflammation. Overall, these findings suggest the possibility of a trained immunity scenario, where $PM_{2.5}$ heightens inflammatory cytokine responses to multiple stimulators, and dampens sensitivity to inhibitors which counter-regulate these responses.

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Air pollution is a substantial contributor to morbidity and mortality. The Global Burdens of Disease Study estimated that in 2019, ambient fine particulate matter ($PM_{2.5}$), defined as particles with aerodynamic diameter $\leq$2.5 μm, was the fourth leading cause of mortality across the globe, contributing to 6.5 million excess deaths (GBD 2019 Risk Factors Collaborators, 2020). Though sustained PM2.5 exposure has long been associated with morbidity and mortality from respiratory conditions, mounting evidence indicates it also forecasts risk for adverse outcomes in the context of multiple autoimmune, cardiometabolic, reproductive,

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neoplastic, and psychiatric diseases (Glencross et al., 2020; Keswani et al., 2022; Schraufnagel et al., 2019b; Yang et al., 2019).

These health consequences are thought to arise from a complex mixture of organ specific and systemic responses to long-term $PM_{2.5}$. However, one pathogenic mechanism that is hypothesized to contribute to the broad array of health problems attributed to $PM_{2.5}$ involves sustained activation of inflammatory signaling (Schraufnagel et al., 2019a; Thangavel et al., 2022). Consistent with this hypothesis, *ex vivo* studies demonstrate that $PM_{2.5}$ acutely upregulates inflammatory activity in airway epithelial cells, alveolar macrophages, and leukocytes from both the innate and adaptive arms of the immune system (Klümper et al., 2015; Liu et al., 2019; Manzano-León et al., 2016; Cachon et al., 2014; Mitkus et al., 2013; Zou et al., 2020; Miyata and van Eeden, 2011). In addition to these direct effects, $PM_{2.5}$ also modifies the way that airway epithelial and innate immune cells respond to subsequent challenges with different stimuli (van Eeden et al., 2001). In fact, two recent epidemiologic studies suggest ambient $PM_{2.5}$ sensitizes innate immune cells, so they mount larger *ex vivo* chemokine and cytokine responses to lipopolysaccharide (LPS), a molecule expressed by Gram-negative bacteria (Tripathy et al., 2021; Raqib et al., 2022). These findings suggest the possibility that $PM_{2.5}$ induces trained immunity (Netea et al., 2020), a broader process where an initial encounter with a stimulus functionally reprograms innate immune cells, altering their responsivity to subsequent challenges from other stimuli. If this is the case, it could explain how the inflammation acutely evoked by $PM_{2.5}$ is sustained over time, and why it disseminates beyond the airways to other bodily tissues, e.g., brain, gut, heart, and contributes to a broad array of health problems (Schraufnagel et al., 2019b; Thangavel et al., 2022).

However, before any such conclusions can be reached, these results must be replicated and extended. Our goal here is to begin this process, first by replicating the relationship between ambient $PM_{2.5}$ and immune sensitization in a new sample of healthy children, and then by answering three questions unresolved in the literature. First, how general is any sensitization effect? The recent studies (Tripathy et al., 2021; Raqib et al., 2022) considered responsivity to LPS, a ubiquitous bacterial stimulus for innate immune cells. However, many other stimuli bearing pathogen-associated and/or danger-associated molecular patterns also stimulate inflammatory activity in these cells by engaging toll-like and NOD-like receptor pathways (Armutcu, 2019). To model these interactions, we examined the relationship between $PM_{2.5}$ and cellular responsivity to LPS, as well as other stimulators that mimic exposure to viruses, tissue damage, and oxidative stress. Since chronic inflammation often results from both excessive responsivity and inadequate termination of an initial response (Nathan and Ding, 2010), we also considered whether $PM_{2.5}$ is associated with dampened sensitivity to agents that promote resolution of inflammation. We expected that higher ambient $PM_{2.5}$ would be associated with larger cytokine responses to stimulators and lower sensitivity to inhibitors, above and beyond individual and household characteristics that might contribute to such relationships, e.g., economic hardship.

Second, there is regional and seasonal variation in the composition of $PM_{2.5}$, which reflects differing mixtures of dozens of constituents, including elemental carbon, ammonium, nitrate, sulfate, and other trace elements. Emerging data suggest these constituents may have differing consequences for immunologic activity and disease vulnerability (Manzano-León et al., 2016; Wang et al., 2023; Nan et al., 2023; Qiu et al., 2022). Thus, we leveraged hyperlocal estimates of 15 major $PM_{2.5}$ constituents at a spatial resolution of 50 m (Amini et al., 2023a, 2023b; 2023c), and apportioned them (Knobel et al., 2023) into five likely emission sources. Because of the relatively high abundance of traffic-related pollution and biomass combustion around Chicago (Zhang et al., 2014), where our sample resided, we hypothesized that $PM_{2.5}$ components typically released by these sources would be most consistently related to inflammatory activity, as reflected in larger cytokine responses to stimulators and lower sensitivity to inhibitors.

Third, we considered durability over time. Both of the earlier reports suggesting that $PM_{2.5}$ induces trained immunity (Tripathy et al., 2021; Raqib et al., 2022) assessed inflammatory activity on a single occasion. Here we used a longitudinal design, where the *ex vivo* studies outlined above were conducted with whole blood leukocytes collected from children during both eighth (ages 12–14) and tenth grade (ages 14–17). The two years between these assessments often represents a period of significant change for children, during which social, emotional, and biological transitions associated with puberty can recalibrate stress-responsive systems (Gunnar and Howland, 2022). Nonetheless, based on indications that trained immunity strengthens with additional exposures (Netea et al., 2020), we predicted that $PM_{2.5}$ associations with cytokine responsivity and inhibition sensitivity would be durable across the follow-up period.

Finally, if high $PM_{2.5}$ reprograms innate immune cells to have larger responses to stimulators and lower sensitivity to inhibitors, this tendency could over time foster systemic inflammation, a contributor to multiple chronic diseases (Nathan and Ding, 2010; Furman et al., 2019). To evaluate this hypothesis, we considered the relationship between $PM_{2.5}$ constituents and biomarkers reflecting this process, including C-reactive protein (CRP), and interleukins (IL)-6, −8, −10, tumor necrosis factor-α (TNF-α) and soluble urokinase-type plasminogen activator receptor (suPAR).

1. Methods

1.1. Sample

The data are from a larger study of socioeconomic disparities in brain development and cardiovascular health (Miller et al., 2018). It involved 277 children from the Chicago area, recruited through ads in media, public transit, and schools. To be eligible, children had to be in eighth grade, English-speaking, and in good health, defined as (a) non-pregnant, (b) without a history of chronic medical or psychiatric/neurological illness, (c) free of prescription medications for the past month, (c) without acute infectious disease for two weeks, and (d) without MRI scanning contra-indications, i.e., metal implants or devices that could pose safety hazards in the magnet. Each child gave written assent to participate, and a parent or guardian gave written consent. Northwestern University's IRB approved the protocol.

Children completed visits in eighth grade and two years later, in tenth grade. At each, they completed surveys and gave fasting antecubital blood, while a parent/guardian provided demographic data. Time 1 visits began in April 2015 and ended in March 2017. Time 2 visits began in June 2017 and ended in June 2019. The mean duration between visits was 24.01 months, SD = 1.48. Of the 277 children enrolled at Time 1, 257 returned for Time 2 (92.78%).

1.2. Ambient particulate matter and constituents

A parent or guardian provided each child's primary residential address, and its longitude and latitude was identified with ArcGIS World Geocoder (all but one address yielded a successful match). Residential $PM_{2.5}$ was estimated for a 50-m grid around those coordinates, with hyperlocal machine-learning (ML) estimates from Amini and colleagues (Amini et al., 2023a,b,c). These models provided annual mean estimates of elemental carbon (EC), organic carbon (OC), ammonium (NH_4^+), nitrate (NO_3^-), sulfate (SO_4^{2-}), iron (Fe), copper (Cu), silicon (Si), calcium (Ca), nickel (Ni), vanadium (V), lead (Pb), zinc (Zn), bromine (Br), and potassium (K) using algorithms that integrated data from 987 monitoring sites, primarily from the US Environmental Protection Agency (EPA). The model also draws on satellite measurements from Google Earth Engine, simulations from chemical transport models, meteorological conditions, and land-use data. Hyperparameter tuning was conducted on a training dataset (70%), and the model was evaluated on a testing dataset (30%). The model performed exceptionally well, with out-of-sample cross-validation R^2 from 0.821 (Br) to 0.975 (SO_4^{2-}).

Household $PM_{2.5}$ mass ($\mu g/m^3$) was estimated by summing values of the 15 constituents over 3 calendar years preceding each child's study entry (in sensitivity analyses 5 years' of data were used). To facilitate comparison with earlier studies, estimates of neighborhood $PM_{2.5}$ ($\mu g/m^3$) were obtained from the EJScreen dataset (Berrocal et al. 2010a, 2010b). EJScreen provides $PM_{2.5}$ at the level of census tracts (downscaled CMAQ model predictions), which typically have 1200–8000 residents. Because EJScreen is updated every few years, we used the dataset from 2014, which was closest in time to when children entered the study.

Drawing on matrix factorization analyses of hyperlocal estimates (Knobel et al., 2023), we apportioned the 15 p.m.$_{2.5}$ constituents into tracer composites reflecting five likely sources: motor vehicles; soil and crustal dust; heavy oil and industrial; metal processing and agriculture; and fuel and biomass combustion (details in online supplement). Like the constituents themselves, these composites ($\mu g/m^3$) reflect average exposures for 3 years prior to study entry, in a 50-m grid around the household.

1.3. Blood collection

Antecubital blood was collected at both visits by a trained phlebotomist. To minimize circadian variation, venipuncture was always performed between 8:00–10:00 a.m. Children abstained from food for 8 h beforehand to minimize dietary influences on inflammatory outcomes.

1.4. Inflammatory responsivity

Whole blood was drawn into Sodium-Heparin Vacutainers and, within an hour, diluted to a 9:1 ratio with R10 media, and cultured with microbial and sterile triggers of inflammation. Specifically, 400 μL aliquots of diluted blood were added to wells containing (a) 50 ng/mL LPS (Invivogen), (b) 1 μg/mL Resiquimod (R848; Invivogen), which triggers anti-viral activity (c) 0.1 μg/mL heat-shock protein-60 (HSP-60; R&D Systems), released following tissue damage; or (d) 20 μg/mL of advanced glycation end-product from bovine-serum albumin (AGE-BSA; BioVision); oxidant found in processed foods and cigarette smoke. To quantify sensitivity to inhibition, 400 μl of diluted blood was added to wells containing 50 ng/mL LPS, plus either (a) hydrocortisone at doses of 2.76×10^{-7} M or 2.76×10^{-6} M, or (b) IL-10 at doses of 1.08×10^{-9}, 5.38×10^{-9}, or 2.69×10^{-8} M. Supernatants were harvested after 6 h of incubation at 37 °C and frozen until the cytokines IL-1β, IL-6, TNF-α and IL-8 were quantified by multiplex immunoassay (Luminex Magpix). Intra-assay coefficients of variation for duplicates ranged from 1.59 to 2.28%, and inter-assay coefficients of variation from 2.67 to 4.03%.

To reduce false discoveries, we conducted primary analyses on two composite endpoints. The first was a stimulation composite, formed by averaging *z*-scored values across the four cytokines (mean pairwise correlation = 0.59), then across the four stimulating agents (mean pairwise correlation = 0.63). The composite was internally consistent (Cronbach's α = 0.87 and 0.85 at Time 1 and 2) and scored so that higher values represent larger cytokine responses to stimulating agents. The second was an inhibition composite. It was formed by estimating a child-specific inhibition slope for each cytokine within each condition (Lam et al., 2022). *Z*-scored values of cytokine slopes were then averaged (mean pairwise correlation = 0.52), and collapsed across inhibition conditions (mean correlation = 0.78). Again, the composite was internally consistent (Cronbach's α = 0.91 and 0.88 at Times 1 and 2) and scored so that higher values represent greater sensitivity to anti-inflammatory compounds. Before composites were calculated, all cytokines were corrected for background production, by subtracting out values from the negative-control well containing R10 alone. Results from principal components analyses supported this approach: in data from both visits, the first dimension explained over 70% of the common variance among cytokines that comprised the stimulation and inhibition composites.

1.5. Systemic inflammatory activity

Blood was collected into a Serum-Separator Tube (Becton-Dickinson) and centrifuged for 10 min at 1200×*g* within an hour of venipuncture. The serum was harvested and divided into aliquots, then frozen at −80 C until the study ended. At that point, we quantified serum levels of six biomarkers reflecting systemic inflammatory activity (Furman et al., 2019; Hodges et al., 2015; Ridker, 2016): C-reactive protein, IL-6, IL-8, IL-10, TNF-α, and suPAR. CRP was measured in duplicate on a Roche/Hitachi cobas c502 instrument. Cytokines were measured in triplicate by immunoassay (Aldo et al., 2016) on an microfluidic platform (Simple Plex; Protein Simple). suPAR was measured in duplicate by immunoassay (Human Quantikine ELISA; R&D Systems). Intra-assay coefficients of variation ranged from 1.6% to 5.0%. Biomarkers were skewed, so we normalized their distributions with log-10 transformations. The logged values were *z*-scored, then averaged into a composite, which exhibited good internal consistency, with Cronbach's α = 0.65 and 0.70 at Time 1 and 2. In principal components analyses, the first dimension explained 39% (Time 1) and 42% (Time 2) of the common variance among biomarkers. At neither timepoint was a robust second dimension evident (eigenvalues <1.05). Still, given the moderate-sized inter-correlations among these indicators of inflammation, we performed exploratory analyses relating $PM_{2.5}$ indices to individual biomarkers.

1.6. Statistical analysis

Covariates. All models included covariates reflecting age (in years), sex at birth (male = 0, female = 1), race/ethnicity (three variables reflecting self-endorsement of Black, Asian, and/or Hispanic identities; coded as Absent = 0, Present = 1 to allow for multiple identities), household income-to-poverty ratio, and pubertal status, assessed with a validated self-report measure (Peterson et al., 1988) ranging from 1 (pre-pubertal) to 5 (post-pubertal). Because the Chicago area has pronounced racial disparities in income, we considered the possibility these covariates might be highly collinear, leading to unstable or imprecise parameter estimates. However, tolerance statistics for covariates related to race, ethnicity, and income-to-poverty ratio were uniformly above 0.71, indicating that multi-collinearity was not a concern here.

Missing Data. For cross-sectional analyses, the analytic sample consisted of 272 children. Of the 277 children enrolled, 1 did not provide a matchable address and 5 were missing inflammation data because of technical difficulties. For longitudinal analyses, the analytic sample consisted of 253 children. Attrition primarily reflected children who were lost to follow-up.

Analytic Plan. Hypotheses were evaluated using linear regression in SPSS 29.0. For each cross-sectional hypothesis, we first estimated a crude model relating $PM_{2.5}$ mass - or its components – to inflammatory responsivity. Then we estimated an adjusted model with the covariates listed below. For longitudinal hypotheses, the same strategy was used, except for in adjusted models we included Time 1 values of the inflammatory outcome. Significance tests were two-tailed, with α = 0.05.

2. Results

2.1. Preliminary analyses

Table 1 describes the sample's characteristics. Fig. 1 depicts the distributions of exposure variables involving $PM_{2.5}$ mass and constituents. On a relative basis, children had the greatest exposure to $PM_{2.5}$ constituents typically emitted by fuel and biomass combustion, followed by motor vehicles, and metal processing and agricultural sources. Fig. 2 shows the spatial distribution of $PM_{2.5}$ in the Chicago area. Exposures were spatially clustered, an observation also apparent from the intercorrelations that are presented in Table S1. Those values indicate that children exposed to high concentrations of motor vehicle emissions

Table 1
Characteristics of the analytic sample at Time 1 (n = 272).

Characteristic	N (%) or Mean (SD)
Age, years	13.92 (0.54)
Sex, female	173 (63.6%)
Self-identify as White	106 (39.0%)
Self-identify as Black	104 (38.2%)
Self-identify as Asian, mixed or other	26 (9.6%)
Self-identify as Hispanic/Latinx	88 (32.4%)
Pre, Early, or Mid Puberty	98 (36.1%)
Late or Post Puberty	174 (63.9%)
Household Income to poverty ratio	3.67 (4.37)
Households in poverty (IPR <1.00)	50 (18.4%)
Low income households (IPR 1.00–1.99)	58 (21.3%)

Note. Children can endorse multiple racial identities, so values in these categories exceed 100 percent.

tended to be co-exposed to $PM_{2.5}$ constituents from other sources. This pattern was particularly evident for constituents from heavy oil and industrial sources ($r = 0.88$), soil and crustal dust ($r = 0.85$), and fuel and biomass combustion ($r = 0.55$). The correlation between residential and neighborhood $PM_{2.5}$ mass was $r = 0.61$, likely reflecting both spatial variability and imprecise estimation (see Discussion). Table S2 presents associations of exposures with covariates. Reflecting broader trends in the US, children from lower-income families, and who identified as Black and/or Hispanic, had higher exposure to residential and neighborhood $PM_{2.5}$ mass and most constituents (Knobel et al., 2023; Colmer et al., 2020; Tessum et al., 2019). Figs. S1–S3 present distributions of inflammation outcomes.

2.2. Stimulated cytokine production and sensitivity to inhibition

Tables 2 and 3 present cross-sectional results. In crude models, children living in residences and neighborhoods with higher $PM_{2.5}$ mass displayed larger cytokine responses to stimulation, and less sensitivity to anti-inflammatory agents. Similar patterns were observed for $PM_{2.5}$ constituents emitted from motor vehicles and soil/crustal dust, but not other sources. These associations were attenuated, but generally remained significant, in covariate-adjusted models. We applied Benjamini and Hochberg's step-up procedure (Benjamini, 2010), which uses adjusted p values to control false discovery. At the customary 10% threshold, all stimulated cytokine associations that were significant in adjusted models remained so. However, the associations for inhibition sensitivity did not survive.

Tables 4 and 5 display prospective results over the two-year follow-up. In crude models, neighborhood $PM_{2.5}$ mass was associated with larger cytokine responses, and both residential and neighborhood $PM_{2.5}$ mass were associated lower inhibition sensitivity. Many of the residential tracer composites also were associated with changes in inflammatory outcomes, however these associations were substantially attenuated with covariate adjustment. None of the prospective associations for stimulated cytokine production survived false-discovery control (Benjamini, 2010) at a 10% threshold. Findings were most robust for inhibition sensitivity. Specifically, over the two-year follow-up, children with higher exposure to residential $PM_{2.5}$ mass - and constituents emitted from motor vehicles and fuel/biomass combustion - became less sensitive to anti-inflammatory agents. Both of these associations remained significant at a 10% false-discovery threshold.

In sensitivity analyses we re-estimated these models, substituting 5-year averages of $PM_{2.5}$ constituents in place of 3-year averages. The pattern of results was highly similar, with coefficients in the same direction and of the same magnitude. In 5/6 analyses, the p value associated with the exposure remained significant.

We ran additional sensitivity analyses to determine if geographical clustering of homes was biasing parameter estimates. Children in the sample lived in 240 distinct block groups. 210 of those block groups contained the home of just one participant, whereas 25 had 2 participants and 5 had 3 participants. To gauge whether this nesting affected results, we re-modeled the neighborhood $PM_{2.5}$ data using Generalized Estimating Equations (GEE), specifying block group as a nesting variable and an exchangeable covariance matrix. The findings were identical to those in the regression models above. In cross-sectional

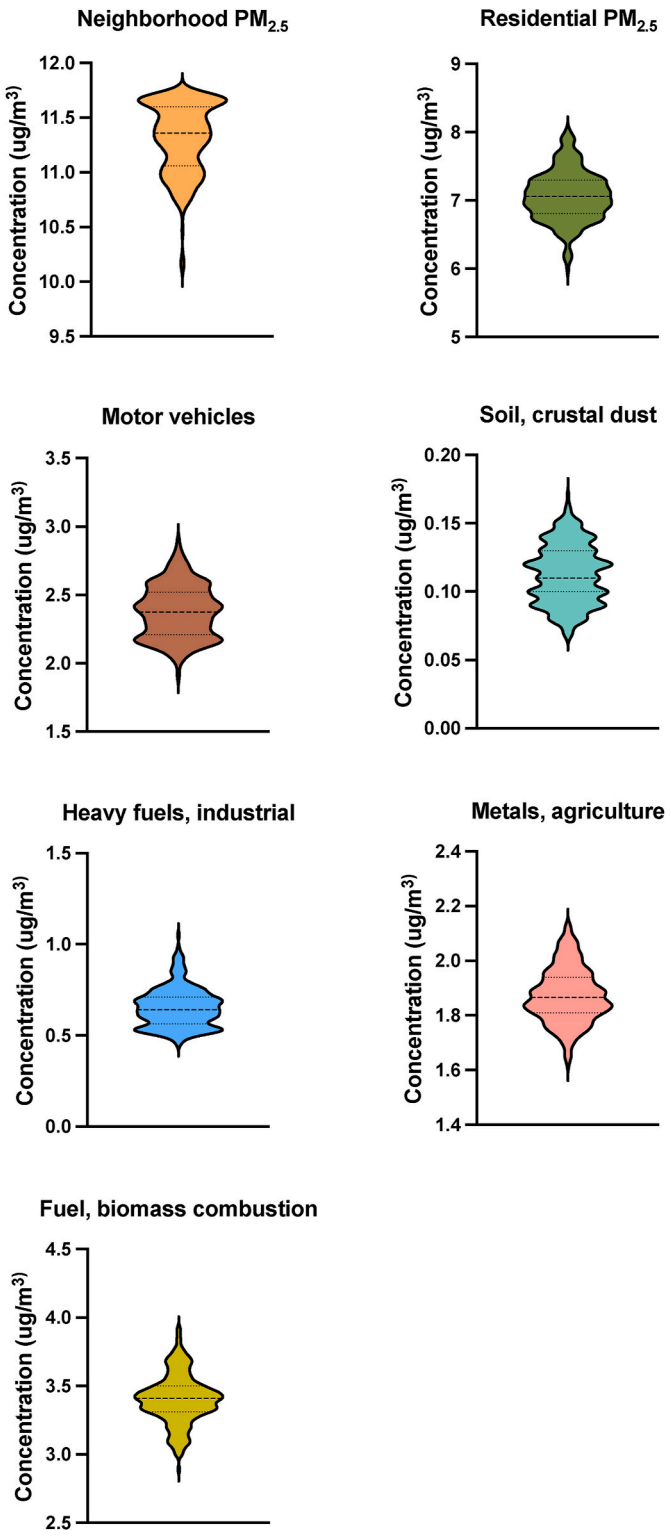

Fig. 1. Distribution of primary exposures. The figures depict violin plots of $PM_{2.5}$ mass and tracer composites at Time 1, when children were in eighth grade. In each figure, the large dashed line is the median value, and the small dashed lines are the 1st and 3rd quartiles of the distribution.

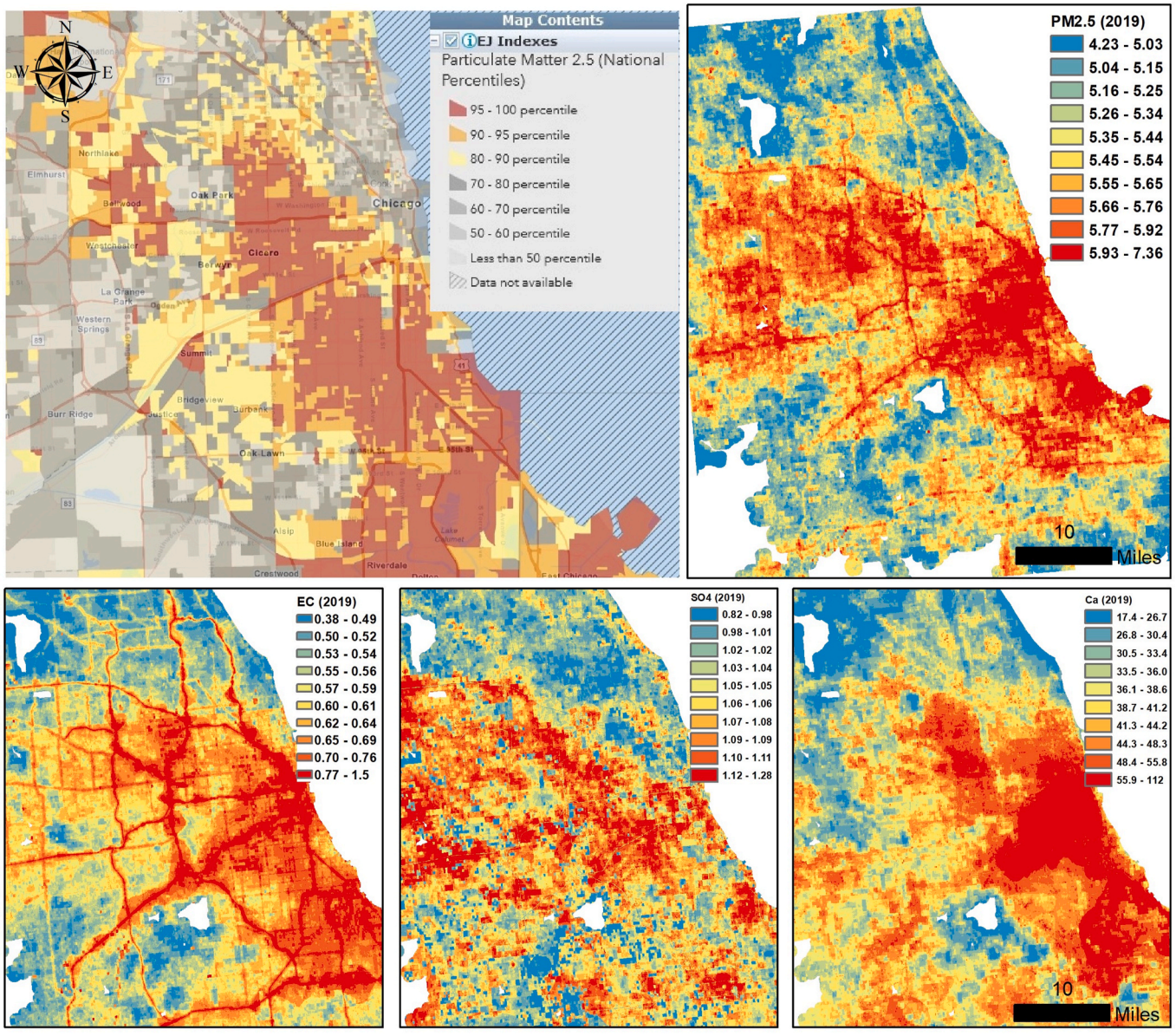

Fig. 2. Long-term mean air pollution predictions for 2019 in Chicago, Illinois. The top-left figure represents neighborhood $PM_{2.5}$ from EJScreen at the block group spatial scale. This data is a downscaled output from the CMAQ model (native 12 km predictions). The top-right figure shows high-resolution (50 m) $PM_{2.5}$ predictions from machine learning ensemble models (sum of 15 p.m.$_{2.5}$ components). The bottom-left map corresponds to elemental carbon (EC), a tracer of motor vehicle emissions. The bottom-middle map represents sulfate (SO_4^{2-}), a tracer of coal burning and power plant emissions. The bottom-right map depicts calcium (Ca), a tracer of soil/dust emissions. The units for $PM_{2.5}$, EC, and SO_4^{2-} are micrograms per cubic meter, while the units for Ca are nanograms per cubic meter.

analyses, children in neighborhoods with higher $PM_{2.5}$ mass displayed larger cytokine responses to stimulation ($p = 0.018$) and less sensitivity to anti-inflammatory agents ($p = 0.050$), but only the former relationship was significant at a 10% false-discovery threshold. Similar to the pattern in regression models, there was no relationship between neighborhood $PM_{2.5}$ and inflammatory composites in longitudinal GEEs.

To clarify which stimuli $PM_{2.5}$ sensitizes immune cells to, we conducted separate analyses of cytokine responses to each stimulating and inhibitory agent (Tables S3 and S4) that survived false-discovery correction. In cross-sectional analyses, neighborhood $PM_{2.5}$ mass and household $PM_{2.5}$ constituents were associated with larger cytokine responses to all four stimulating agents, with minor variability in the significance of these associations. In prospective analyses, residential $PM_{2.5}$ mass and constituents from motor vehicles and fuel/biomass combustion were associated with declining sensitivity to inhibition from both interleukin-10 and glucocorticoids.

2.3. Circulating inflammatory biomarkers

The results of models focused on circulating inflammatory biomarkers are presented in Tables S5 and S6. As these tables indicate, these associations were non-significant. To determine whether the composite was masking any associations, we conducted exploratory analyses relating $PM_{2.5}$ indices to individual biomarkers. While a handful of these associations reached nominal levels of significance ($p < 0.05$), none of them survived 10% false-discovery corrections.

3. Discussion

Recent analyses suggest that high levels of ambient $PM_{2.5}$ induce trained immunity (Tripathy et al., 2021; Raqib et al., 2022), which may help explain the excess morbidity and mortality from diseases involving chronic inflammation in polluted areas (Schraufnagel et al., 2019a;

Table 2
Results of cross-sectional linear regression models predicting stimulated cytokine release.

Exposure	Model	B	95% CI	p
Neighborhood $PM_{2.5}$	1	0.45	+0.14, +0.77	0.005
	2	0.41	+0.08, +0.74	0.015
Residential $PM_{2.5}$	1	0.28	+0.00, +0.55	0.047
	2	0.15	−0.12, +0.43	0.280
Residential motor vehicle	1	0.71	+0.19, +1.23	0.008
	2	0.66	+0.14, +1.19	0.014
Residential soil, crustal dust	1	6.36	+1.66, +11.06	0.008
	2	6.63	+1.82, +11.45	0.007
Residential heavy fuel, industrial	1	0.86	−0.08, +1.80	0.071
	2	1.02	+0.09, +1.95	0.031
Residential metal processing, agriculture	1	0.73	−0.29, +1.74	0.159
	2	0.68	−0.30, +1.67	0.174
Residential fuel, biomass combustion	1	0.48	−0.08, +1.04	0.093
	2	0.08	−0.49, +0.64	0.790

Note: Model 1 is crude; cytokine release is regressed on $PM_{2.5}$ mass or tracer composite alone. Model 2 is adjusted; it also includes variables reflecting age, sex, race and ethnicity, pubertal status, and income-to-poverty ratio. Neighborhood $PM_{2.5}$ is estimated for the census tract where child's primary residence is located. Residential exposures are estimated for a 50-m grid surrounding that residence.

Table 3
Results of cross-sectional linear regression models predicting sensitivity to inhibition.

Exposure	Model	B	95% CI	p
Neighborhood $PM_{2.5}$	1	−0.46	−0.82, −0.10	0.012
	2	−0.33	−0.69, +0.02	0.072
Residential $PM_{2.5}$	1	−0.26	−0.57, +0.06	0.111
	2	−0.04	−0.34, +0.26	0.779
Residential motor vehicle	1	−0.73	−0.1.32, −0.13	0.017
	2	−0.56	−1.14, +0.00	0.050
Residential soil, crustal dust	1	−6.29	−11.66, −0.92	0.022
	2	−5.65	−10.90, −0.38	0.035
Residential heavy fuel, industrial	1	−0.72	−1.79, 0.35	0.185
	2	−0.79	−1.80, +0.22	0.126
Residential metal processing, agriculture	1	−0.46	−1.61, +0.70	0.435
	2	−0.36	−1.44, +0.72	0.515
Residential fuel, biomass combustion	1	−0.50	−1.14, +0.13	0.121
	2	+0.11	−0.51, +0.72	0.732

Note: Model 1 is crude; inhibition sensitivity is regressed on $PM_{2.5}$ mass or tracer composite alone. Model 2 is adjusted; it also includes variables reflecting age, sex, race and ethnicity, pubertal status, and income-to-poverty ratio. Neighborhood $PM_{2.5}$ is estimated for the census tract where child's primary residence is located. Residential exposures are estimated for a 50-m grid surrounding that residence.

Thangavel et al., 2022). Here we replicated the primary findings of these studies in healthy children, observing that higher neighborhood $PM_{2.5}$ mass covaried with larger inflammatory cytokine responses to *ex vivo* stimulation with LPS. This pattern was evident even after models were adjusted for children's racial identity and household income, which in the US, are strongly related to the distribution of $PM_{2.5}$ and likelihood of health problems (Knobel et al., 2023; Colmer et al., 2020; Miller et al., 2011; Boyce et al., 2021). These results are consistent with the hypothesis that ambient $PM_{2.5}$ acts as a sensitizing agent, which functionally programs innate immune cells to respond more aggressively to LPS, a ubiquitous bacterial stimulus in contemporary society.

To evaluate the generality of this phenomenon, we also quantified cytokine responsivity to other stimulators, and to inhibitors that promote the resolution of inflammation. In cross-sectional analyses, higher neighborhood $PM_{2.5}$ mass covaried with larger cytokine responses to

Table 4
Results of prospective linear regression models predicting stimulated cytokine release.

Exposure	Model	B	95% CI	p
Neighborhood $PM_{2.5}$	1	0.57	+0.25, +0.89	0.001
	2	0.22	−0.06, +0.49	0.123
Residential $PM_{2.5}$	1	0.47	+0.20, +0.75	0.001
	2	0.26	+0.03, +0.48	0.026
Residential motor vehicle	1	0.93	+0.40, +1.46	0.001
	2	0.39	−0.05, +0.83	0.084
Residential soil, crustal dust	1	7.24	+2.45, +12.03	0.003
	2	2.13	−1.95, +6.21	0.305
Residential heavy fuel, industrial	1	1.26	+0.31, +2.21	0.010
	2	0.63	−0.14, +1.41	0.110
Residential metal processing, agriculture	1	1.38	+0.35, +2.40	0.009
	2	0.81	−0.01, +1.61	0.052
Residential fuel, biomass combustion	1	0.74	+0.17, +1.31	0.011
	2	0.36	−0.10, +0.83	0.126

Note: Model 1 is crude; cytokine release is regressed on $PM_{2.5}$ mass or tracer composite alone. Model 2 is adjusted; it also includes variables reflecting age, sex, race and ethnicity, pubertal status, income-to-poverty ratio, and cytokine release at Time 1. Neighborhood $PM_{2.5}$ is estimated for the census tract where child's primary residence is located. Residential exposures are estimated for a 50-m grid surrounding that residence.

Table 5
Results of prospective linear regression models predicting sensitivity to inhibition from tracer composites.

Exposure	Model	B	95% CI	p
Neighborhood $PM_{2.5}$	1	−0.43	−0.80, −0.06	0.022
	2	−0.09	−0.39, +0.22	0.579
Residential $PM_{2.5}$	1	−0.56	−0.88, −0.25	0.000
	2	−0.38	−0.62, −0.14	0.002
Residential motor vehicle	1	−0.99	−0.1.59, −0.39	0.001
	2	−0.52	−1.00, −0.04	0.034
Residential soil, crustal dust	1	−7.92	−13.37, −2.47	0.005
	2	−3.71	−8.13, +0.70	0.099
Residential heavy fuel, industrial	1	−1.19	−2.27, −0.10	0.032
	2	−0.80	−1.64, +0.04	0.062
Residential metal processing, agriculture	1	−0.56	−1.74, +0.62	0.347
	2	−0.20	−1.09, +0.69	0.661
Residential fuel, biomass combustion	1	−1.21	−1.84, −0.57	0.000
	2	−0.83	−1.33, −0.33	0.001

Note: Model 1 is crude; inhibition sensitivity is regressed on $PM_{2.5}$ mass or tracer composite alone. Model 2 is adjusted; it also includes variables reflecting age, sex, race and ethnicity, pubertal status, income-to-poverty ratio, and inhibition sensitivity at Time 1. Neighborhood $PM_{2.5}$ is estimated for the census tract where child's primary residence is located. Residential exposures are estimated for a 50-m grid surrounding that residence.

other agents, including those which mimic tissue damage (HSP-60) and oxidative stress (AGE-BSA), both of which are common triggers of the sterile inflammation thought to promote aging-related disease (Furman et al., 2019). In prospective analyses spanning two years, children with higher residential $PM_{2.5}$ exposure also became less responsive to inhibitory agents released locally (IL-10) and systemically (glucocorticoids). Together, these findings suggest the hypothesis that $PM_{2.5}$ has more general effects on the responsivity of innate immune cells, rendering them *more* sensitive to agents that provoke inflammation, and *less* sensitive to agents that suppress it. These associations are small in magnitude, with correlations in the 0.10-0.20 range after covariate adjustment, and there is variability how strongly specific $PM_{2.5}$ exposures relate to specific inflammatory outcomes. Nevertheless, the consistency apparent in stimulator- and inhibitor-specific analyses (Tables S3 and S4) suggests a general pattern indicative of trained immunity.

With that said, we acknowledge the sensitization findings are inconsistent with some experimental studies (Manzano-León et al., 2016; Klümper et al., 2015; Hirota et al., 2015), which have observed that *in vitro* exposures to particulate matter can dampen inflammatory responses to subsequent stimuli. These discrepant patterns may reflect differences in the coarseness of particles ($PM_{2.5}$ vs. PM_{10}), duration of exposure (acute vs. chronic), or setting (laboratory vs. naturalistic), as well as the immunologic target (fresh primary leukocytes vs. cultured alveolar macrophages vs. immortalized cell lines) (Miyata and van Eeden, 2011; Ferguson et al., 2013).

To determine whether specific constituents of $PM_{2.5}$ might underlie trained immunity, we considered how emissions from five likely sources related to inflammatory responsivity. In cross-sectional analyses, higher exposure to motor vehicles and soil/crustal dust constituents related to larger cytokine responses to stimulators, but none of the tracer composites covaried with inhibition sensitivity. The opposite pattern emerged in prospective analyse: Higher exposure to constituents from motor vehicles and fuel/biomass combustion forecast declining sensitivity to inhibitory agents, but were unrelated to stimulator responses. Together, these observations suggest two tentative conclusions. The first is that $PM_{2.5}$ modulates cellular responsivity to stimulators and inhibitors over different timescales, with the former effect manifesting more proximally to exposure and the latter more distally. Because we do not have a ready mechanistic explanation for this pattern, it should be considered preliminary until independently replicated and better understood. A second tentative conclusion is that of the emission sources considered, motor vehicles had the most consistent associations with inflammatory outcomes, relating to both stimulator responsivity and inhibition sensitivity, even after false-discovery adjustments. Whether this specificity is unique to the Chicago area or more general remains to be determined.

Finally, we sought to determine whether the contemporaneous associations in earlier studies (Tripathy et al., 2021; Raqib et al., 2022) would be durable across the two-year follow-up, and manifest in systemic inflammatory activity. The results of the durability analyses were ambiguous. We observed cross-sectional associations between multiple $PM_{2.5}$ indicators and stimulator responsivity, however these relationships declined in magnitude and became non-significant at follow-up. By contrast, significant associations between $PM_{2.5}$ indicators and inhibition sensitivity did not materialize until the second assessment, suggesting they became stronger during follow-up. Developmentally, the two-year interval between assessments coincided with major social, emotional, and biological transitions for many children. These puberty-related transitions can recalibrate the responsivity of systems activated by threat, e.g., the autonomic nervous system, hypothalamic pituitary adrenocortical axis, and corticolimbic circuitry (Gunnar and Howland, 2022), and our results may reflect that phenomenon in innate immune cells.

Contrary to hypotheses, we did not observe any relationship between $PM_{2.5}$ indicators and systemic inflammatory biomarkers. Because our sample consisted of healthy children, who displayed low concentrations of these biomarkers, the null findings could be attributable to range restriction. An alternative is that these consequences will become evident with time; i.e., that any $PM_{2.5}$-induced changes in stimulator responsivity or inhibition sensitivity take additional time to manifest in systemic inflammation, particularly in a sample as young and healthy as ours. While plausible, Tripathy and colleagues (Tripathy et al., 2021) observed a similar pattern in midlife adults, where ambient $PM_{2.5}$ was associated with larger *ex vivo* cytokine responses to LPS, but not with circulating CRP or IL-6. Together, these results suggest that $PM_{2.5}$ exposure has different consequences for stimulated vs. circulating biomarkers of inflammation. Divergent effects are biologically plausible – the cytokines assayed in *ex vivo* paradigms are produced exclusively by leukocytes, whereas those measured in circulation are likely derived from a mixture of immune, adipose, skeletal, airway, intestinal, and vascular sources (Armutcu, 2019). With that said, most of the evidence for long-term health consequences of inflammation comes from studies of circulating biomarkers (Furman et al., 2019; Hodges et al., 2015; Ridker, 2016), which raises questions about the clinical significance of the patterns seen here. At this point, longer-term follow-up studies of samples like this are required to determine if, as hypothesized above, $PM_{2.5}$-induced changes in stimulator responsivity or inhibition sensitivity manifest in systemic inflammatory activity later in development.

When interpreting our results, several limitations must be considered. First, the observational design precludes inferences about whether $PM_{2.5}$ has causal effects, or is simply acting as a proxy for a different exposure we did not satisfactorily adjust for or consider here. With that said, the patterns observed here are consistent with experimental studies that demonstrate the biological plausibility of $PM_{2.5}$ inducing trained immunity (Miyata and van Eeden, 2011). Second, our estimates of $PM_{2.5}$ are likely to contain measurement error, because they don't capture exposures outside of children's neighborhoods, e.g., in school. Also, we lack data on how long children lived at their current address, and whether they moved to a different location after study entry. These omissions would probably introduce noise into our exposure variables, reducing power to detect hypothesized associations. Third, our estimates of residential $PM_{2.5}$ mass were imprecise. Although computed using hyperlocal concentrations of 15 components, these constituents together account for ~80–90% of $PM_{2.5}$ mass. Since we do not have reliable estimates of the remaining constituents, e.g., dust and sea salt aerosol, our analyses likely underestimate the strength of the associations between $PM_{2.5}$ mass and inflammatory activity. This imprecision also may have contributed to the moderate-sized correlation between residential and neighborhood $PM_{2.5}$ mass ($r = 0.61$), and these variables' somewhat discrepant relationships with inflammatory outcomes. Fourth, the stimulation and inhibition composites that served as outcome variables were strongly inter-related (at Time 1, $r = -0.79$; at Time 2, $r = -0.81$), reflecting the fact that both are calculated using cytokine responsivity to LPS. As a consequence, these outcomes should not be considered fully independent. However, in the present analyses, we do not see this as a cause for major concern, because after false-discovery corrections, none of the $PM_{2.5}$ variables was significantly associated with both composites. Fourth, our sample size was relatively small, and localized to the region around Chicago, so it will be important to evaluate its replicability and generalizability in larger representative cohorts. Finally, because the sample consisted of healthy children, we are not able to evaluate clinical implications of the immunologic findings.

Despite these limitations, our study with high-quality individual data replicates earlier results suggesting that $PM_{2.5}$ induces trained immunity, and extends those results in multiple regards. In doing so, our study helps answer outstanding questions related to the durability of this phenomenon, its generalizability across stimuli, and the contribution of specific $PM_{2.5}$ constituents.

CRediT authorship contribution statement

Gregory E. Miller: Writing – review & editing, Writing – original draft, Supervision, Resources, Funding acquisition, Conceptualization. **Veronica Passarelli:** Writing – review & editing, Writing – original draft, Data curation. **Edith Chen:** Writing – review & editing, Methodology, Funding acquisition, Conceptualization. **Itai Kloog:** Writing – review & editing, Resources, Methodology, Data curation. **Rosalind J. Wright:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Heresh Amini:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence

the work reported in this paper.

Data availability

The authors do not have permission to share data.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envres.2024.118964.

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